STUDIES ON THE FORM OF THE AMPHIBIAN RED BLOOD CELL

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To a student of cell form the erythrocyte is an ideal subject for investigation. It is a free cell, not permanently involved in contact with other cells, and it has a definite and relatively simple form. I recently published an account of a model which was proposed as a partial explanation for the elliptical form of the amphibian red cell (Davison, 1957). Since the model has served as a guide to the present work, I will briefly describe its salient features as an introduction to these further observations.

The blood cells of the newt *Triturus viridescens* approximate thin elliptical discs in form. Viewed as plane elliptical figures, triploid cells have approximately 1.5 times greater area than diploid blood cells, but are apparently no greater in thickness, a relationship similar to that described by Fankhauser for 2n and 3n skin epidermal cells (Fankhauser, 1952). Not only are the 3n cells larger, they clearly have a different shape than 2n cells, being more eccentric regarded as elliptical figures. Using the ratio of the major to minor axes (a/b) as an index to cell form, 2n and 3n *Triturus* red cells were found to have mean eccentricities of 1.55 and 1.82, respectively.

It has long been recognized that liquid drops can, under the proper physical conditions, simulate many protoplasmic structures (Thompson, 1942). Reasoning that the blood cell exists in a system of cylinders, the blood vessels, I thought it might prove interesting to examine the form characteristics of a fluid drop in contact with a cylindrical surface. If one places a large (29 cm. in diameter) cylindrical glass vessel with the axis horizontal, and pours mercury on the inside of the cylinder, the mercury will assume the form of a flat elliptical disc. Adding more mercury to the pool increases both the area and the eccentricity of the drop but does not appreciably increase its thickness. The model thus simulates the form differences observed between 2n and 3n blood cells. In the model the mercury is in contact with the cylindrical surface through the deforming force of gravity. In the animal it is clear that the blood cells are applied to the wall of the capillary but are not so oriented during their passage through larger vessels. No significant differences were found in the diameter of 2n and 3n capillaries, an essential point, since it is also clear from the model that the larger the cylinder the less eccentric the fluid drop. The latter observations from the model suggest that changes in capillary diameter should lead to alterations in red cell form, with an increase in

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cell eccentricity following a decrease in capillary diameter and a decrease in cell eccentricity following an increase in capillary diameter.

With this background in mind, the further objectives of the study may be stated as follows:

(1) To examine cell form when expressed as a continuous function of cell area, especially with reference to the cross-sectional area of the capillary.

(2) To examine the effect of changes in capillary diameter on red cell form under conditions of constant cell area.

(3) To quantitatively relate these variables.

Animals and Methods

Since both diploid and triploid spanish newts (*Pleurodeles waltlii*) were available, this animal was selected to examine cell eccentricity as a function of cell area. *Pleurodeles* cells are less eccentric than those of *Triturus*, better permitting an analysis of the manner in which the blood cell approaches the circular form. The studies on adult *Triturus* followed the accidental discovery that cold-adapted (8.5° C.) animals have much more eccentric blood cells than the same animals maintained at room temperature (air conditioned 21° C.). Also one can conveniently measure capillary diameter in the tail fin of adult *Triturus*, especially the males, while this is not possible in the heavily pigmented *Pleurodeles* adult. Capillary visibility is good in the larvae of both species.

The animals were maintained either singly in small finger bowls or in groups of 5 to 6 in large finger bowls, and fed with beef liver or live *Tubifex*. The cold-adapted *Triturus* had been kept for several months in stainless steel trays in the refrigerator and fed weekly on live *Tubifex* while at room temperature for a few hours.

Experimental procedures were essentially identical for all animals as follows: Blood was obtained by removing about 1 mm. of the tail tip with a pair of scissors and permitting the tail to bleed directly into a drop of buffered saline on a glass slide. The slide was examined immediately without coverslip and the outlines of about 35 cells traced by means of the *camera lucida*. Placing a coverslip on the preparation resulted in a certain amount of deformation so the practice was abandoned in favor of working quickly before any appreciable drying could take place. Certain precautions that were taken should be mentioned. The slide should be very clean to prevent deformation due to adhesion between the red cell and the glass surface. All margins of the cell must come into focus at the same focal setting, indicating that the cell is resting on one elliptical surface and not oriented at an angle to the plane of observation. Following the tracing of a known linear dimension from a stage micrometer it was possible to determine both the area and the eccentricity of the red cell (area $= \frac{1}{4}\pi ab$, and eccentricity $= a/b$, with a and b the major and minor axes of the cell, respectively). A phosphate-buffered saline (pH 7.4) was found to be a suitable medium for the cells, 0.7% NaCl being approximately isotonic for *Pleurodeles* and adult *Triturus* while 0.6% NaCl was more nearly isotonic for larval *Triturus*.

Capillary measurements were made by lightly anesthetizing the animal by short term exposure to 0.1% chloretone solution, rinsing in tap water and placing the

animal on its side on a 5-inch square glass plate. Measurements of capillary diameter were made on the tail fin margin by means of a calibrated ocular micrometer at about 430 ×. Capillaries were identified as the smallest blood vessels constituting a uniform size class when a given portion of the circulation was traced from the arterial to the venous end, and through which the red cells pass in single file. It is important that anesthesia be light as considerable capillary collapse can occur in animals with partially arrested circulation. Measurements were restricted to vessels through which blood was flowing in normal fashion. The measurements on adult *Triturus* were carried out largely on males, not because of any sex differences but because of better visibility in the broader tail fin of the male. The animals recovered from anesthesia in about one hour.

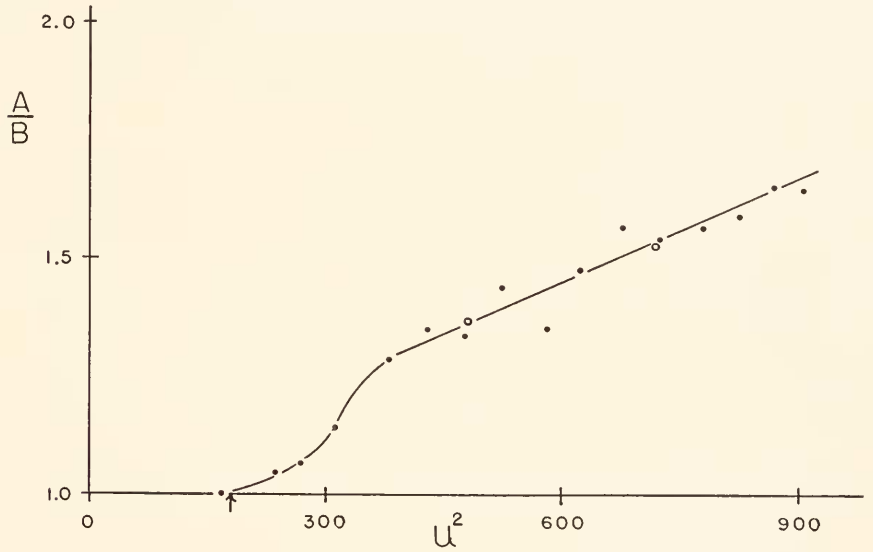

FIGURE 1. The relationship between cell area (μ^2) and cell eccentricity (a/b) in diploid and triploid *Pleurodeles*. The open circles represent the mean values for diploid and triploid blood cells. Other points were obtained by breaking the total sample into classes of 50 μ^2 and plotting the mean values for area and eccentricity within each class. The arrow indicates the mean value for the cross-sectional area of the capillary of the tail fin.

The relationship between red cell form and area was determined as follows with mature larvae of *Pleurodeles*. Approximately 150 cells from diploid animals and an equal number of cells from triploid animals were traced and the eccentricity and area determined for each cell. The mean values for 2n and 3n blood cells were determined from these samples. An additional 30 selected small cells and 30 selected large cells were measured in order to extend the analysis over the widest possible range. The total sample was then arranged in order of increasing cell area, and broken into size classes of 50 μ^2. Within each size class the mean cell area and eccentricity were calculated. Eccentricity (a/b) was then plotted versus area for each size class together with the mean values for 2n and 3n blood (Fig. 1). No significant differences were found between 2n and 3n capillary diameter and

the average capillary cross-sectional area for about 40 determinations is indicated by the arrow in Figure 1.

Triturus came from two sources. The males used for the temperature studies and the larvae represent stock originally from Farmville, Virginia. A small group of female animals of uncertain origin were found to have less eccentric blood cells than those of Farmville animals maintained at the same temperature. These females were unusually large and probably represent a genetically distinct population. In that regard it is interesting to note that the values reported for eccentricity in Missouri animals are different from any of the findings in the present study (Davison, 1957). Analyses were made of cell form, cell area, and capillary cross-sectional area for each of the following groups of animals: Farmville males at 21° C., Farmville larvae at 21° C., females of uncertain origin at 21° C., Farmville males adapted to 8.5° C., and the same males during the adaptation period following transfer to 21° C. From 4 to 10 animals were measured from each group. Blood cell findings represent the means of from 90 to 200 measurements and mean capillary

TABLE I*

Source	Temp. ° C.	Cell area μ^2	Capillary area μ^2	a/b	K in the expression $(a/b)-1=K(A_{cell}/A_{cap.})$
Triturus					
Farmville males	21	583	149	1.69	0.177
Farmville males	8.5	576	113	1.99	0.195
Farmville males	21 (6 days)	590	170	1.64	0.184
Farmville larvae	21	523	211	1.44	0.178
? females	21	579	242	1.39	0.163
Pleurodeles					
Mature larvae (2n)	21	482	184	1.36	0.138
Mature larvae (3n)	21	720	184	1.52	0.134

* Standard deviations for cell area were uniformly about 20% of the mean and about 10% of the mean for capillary diameter and a/b.

size was calculated from 40 to 100 measurements. The same animals were used for capillary and blood cell analyses. The time course experiment following transfer from 8.5 to 21° C. was carried out on a group of 6 *Triturus* on which daily measurements were made for a period of 6 days. The pertinent tabular data derived from these studies appear in Table I.

RESULTS

Cell eccentricity as a function of cell area is plotted in Figure 1. For values of a/b greater than about 1.3, a/b is essentially linear with respect to area and would pass through the origin if extrapolated. For values of a/b less than 1.3, eccentricity rapidly approaches 1 (circular form) as the area of the cell approaches the mean cross-sectional area of the capillary (indicated by the arrow in Figure 1). It is reasonable that if the red cell is no larger than the capillary it can pass through without deformation, accounting for the circular form of the smallest blood cells. Somewhat larger cells may be deformed as they pass through but not actually applied to the wall of the capillary, an interpretation which may account for the

curvilinear portion of Figure 1. Still larger cells slide through the capillary with one elliptical surface applied to the capillary wall, with their form determined in accordance with the linear portion of Figure 1. The graphic information in Figure 1 may be given a somewhat more intuitive presentation as a series of forms in Figure 2. The central circle represents the capillary area (184 μ^2) while the surrounding blood cell forms were constructed from the data of Figure 1 at areas of 300, 500, 700 and 900 μ^2. In this and all other reconstructed cell forms the blood cells were assumed to be perfect ellipses in plane view and were first constructed on coordinate paper employing the basic property of ellipses that $x^2/a^2 + y^2/b^2 = 1$ (a and b are the major and minor semi-axes of the ellipse).

Figure 3 illustrates the reconstructed form of larval and adult Farmville *Triturus* cells and capillaries. The larval blood cell is slightly smaller and less eccentric while the larval capillary is larger.

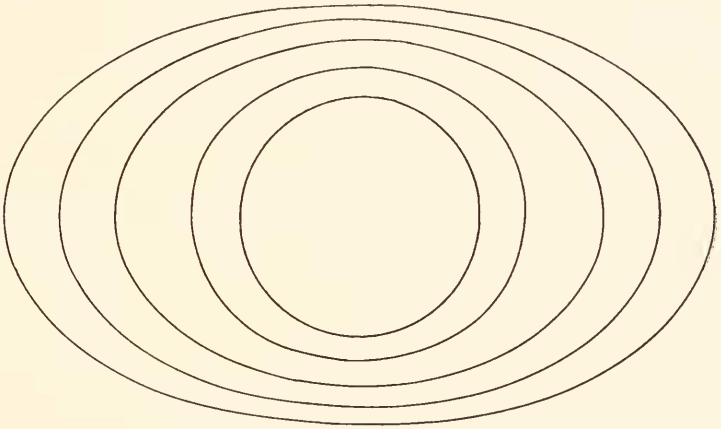

FIGURE 2. The information in Figure 1 was used to reconstruct the form of the blood cells employing the property that $x^2/a^2 + y^2/b^2 = 1$. The central circle is the capillary (184 μ^2). The other figures represent cell forms at 300, 500, 700, and 900 μ^2. The lower line represents 10 μ.

Figure 4 indicates the form differences observed in *Triturus* males maintained at 8.5° C. (left) and 21° C. (center). The right hand figure illustrates red cell form and capillary size in *Triturus* females of uncertain origin (21° C.). It is clear from both Figures 3 and 4 that the larger the capillary the less eccentric the blood cell, a result previously suggested from considerations of the model system. The product of $(a/b) - 1$ and capillary cross-sectional area ($A_{\text{cap.}}$) approximates constancy for adult *Triturus* in which cell area (A_{cell}) is essentially constant. That is:

$$(a/b) - 1 = k/A_{\text{cap.}}$$

The study of eccentricity versus cell area in *Pleurodeles* indicates for the linear portion of the curve (a/b greater than 1.3) that:

$$(a/b) - 1 = k'A_{\text{cell}}.$$

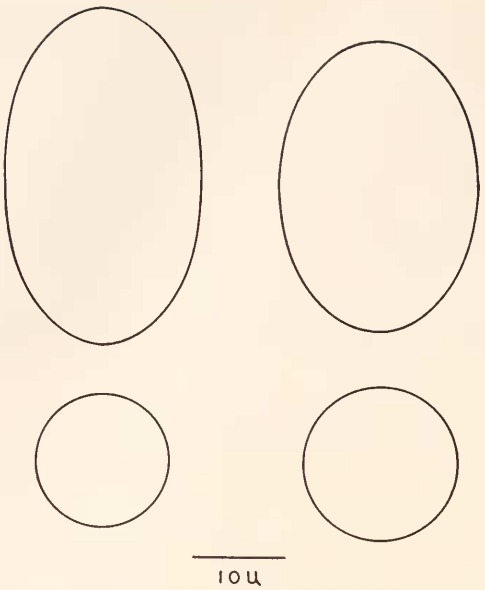

FIGURE 3. The form of the blood cells and capillaries of adult (left) and larval (right) Farmville *Triturus* reconstructed from the tabular data in Table I. The larval blood cell is slightly smaller.

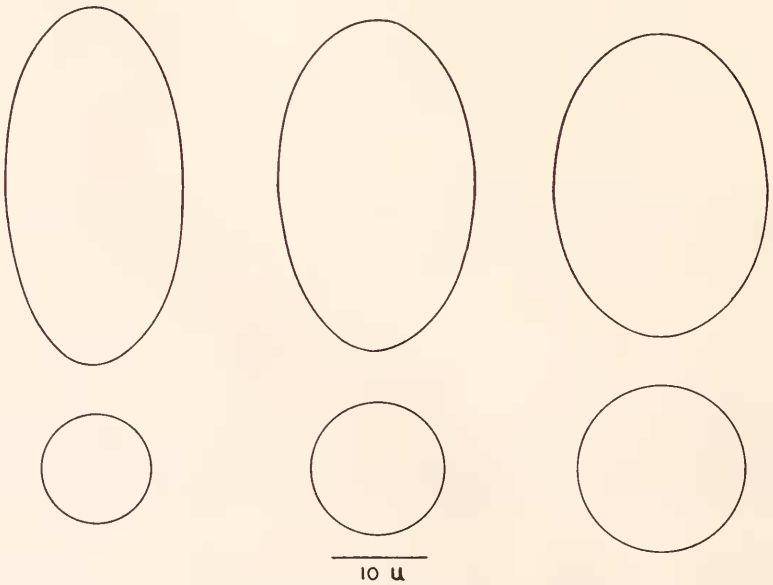

FIGURE 4. The mean forms of blood cells and capillaries in *Triturus* adults constructed from the data in Table I. Farmville males at 8.5° C. (left), Farmville males at 21° C. (center), and females of unknown origin at 21° C. (right). All cells have approximately the same area.

These two expressions may be combined to give an equation relating all three variables: a/b, A_{cell}, and $A_{cap.}$:

$$(a/b) - 1 = K(A_{cell}/A_{cap.}).$$

The extent to which this equation adequately describes the relationship between these variables is clear from the uniformity of the constant K calculated from the data in Table I. *Triturus* values vary from 0.163 to 0.195, while the *Pleurodeles* values are somewhat smaller being 0.138 and 0.134 for diploids and triploids, respectively.

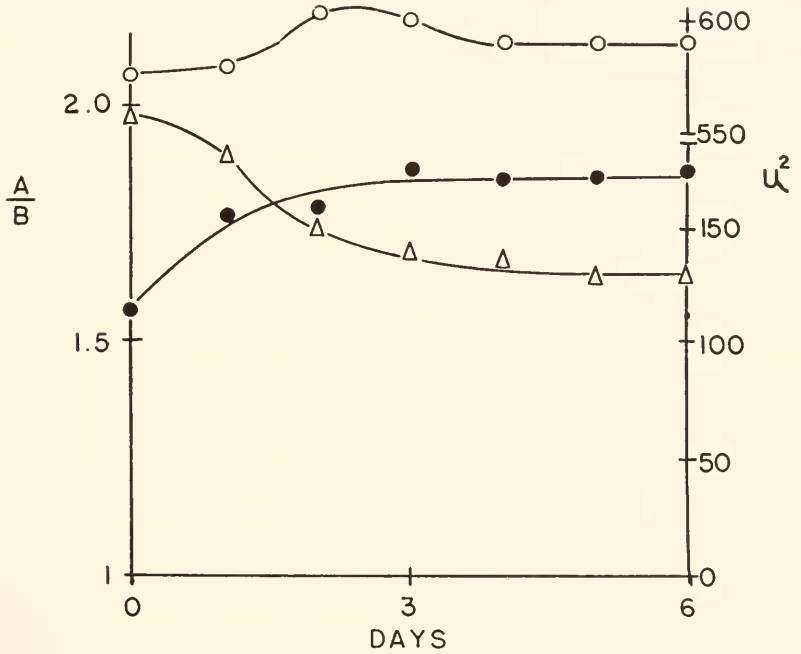

FIGURE 5. The time course of the adaptation from 8.5° C. to 21° C. in Farmville male *Triturus*. Closed circles are capillary cross-sectional area measurements and open circles red cell area measurements (right ordinate). Triangles are eccentricities of the red cells (a/b) (left ordinate). Notice the break in the right ordinate (μ^2).

A point of considerable interest is the time at which red cell form is determined. Two possibilities might be considered. The form of the cell might be determined at the time it first enters the circulatory system with subsequent changes in the capillary environment having no further effect on cell form. If this were true, average cell form should change slowly following a change in capillary diameter, with the total time period for the change equal to the life span of the erythrocyte. On the other hand, if red cell form is plastic, one might expect a more rapid response in red cell form following a change in capillary diameter, a result which would support the concept that red cell form is constantly subject to the forces acting on the cell during its passage through the capillaries. The latter view is

clearly favored by the time course data represented in Figure 5. Following transfer of the animals from 8.5 to 21° C., the increase in capillary diameter is complete within 3 days, while the change in red cell form is largely complete within the same period of time. There is, however, a clear lag in the cell form response and a somewhat greater time for the complete form transformation. It is interesting to note that there is a small transient increase in cell area corresponding to the time period when cell form is most rapidly changing. This increase may represent a temporary change in the osmotic properties of the cell dependent on alterations of the cell surface.

Discussion

The quantitative and temporal relationship between red cell form and capillary size clearly supports the concept that the form of the cell is determined, at least in part, by the size of the capillary through which the cell is passing. Alterations in capillary diameter lead to changes in cell form with kinetics supporting a view that the red cell form is plastic and not fixed. Since the reasoning leading to these analyses was influenced by considerations of the model system, a comparison between the model and the biological systems may be useful. I would like to point out first, however, that model systems serve only to guide rationale, and certainly should not be taken as literal representations of biological reality.

(1) There are gross differences between the relative sizes of the "capillary" and the "cell." In the model the cylinder is many times larger than the mercury drop. In the living system the cell is elliptical only if its area exceeds that of the capillary cross-section, while in the model eccentricity steadily increases with drop area since the drop is always in contact with the cylindrical surface.

(2) There is abundant evidence that the interior of the amphibian cell is fluid like the mercury drop. Norris studied the manner in which displaced nuclei returned to the center of the cell and concluded that except for the nucleus the cell interior is liquid with the shape of the cell conferred by an outer envelope (Norris, 1939). Dawson presumed a liquid interior based on the observation of Brownian movement in the cell interior (Dawson, 1928). Based on microsurgical findings, Seifriz described the cell interior as essentially liquid with a plastic and elastic cell envelope approximately 0.8 μ in thickness (Seifriz, 1926). The envelope thickness agrees well with more recent estimates based on polarized light analyses of human red cells (Mitchison, 1953).

(3) The model system is static while the living system is of course dynamic since the cells are constantly moving through the circulatory system. It is of interest to note that although the blood cells are ellipses with symmetrical ends as observed at rest, they clearly do not have this form while passing through the capillaries. The advancing end of the cell is more rounded than the trailing end so that if the cell could be removed and flattened it would approximate a pear shape in plane view. This configuration may also be imitated with the model by tipping the cylinder and permitting the mercury drop to slowly flow along the surface of the cylinder. The dynamic form of the blood cell may be interpreted as further evidence for a fluid red cell interior.

In summary, then, it appears that red cell form is a consequence of physical forces operating between the cell and the capillary wall. In answer to the classical dilemma of whether form determines function or function determines form one may

arrive at the inadequacy of either of these alternatives and conclude on the basis of these studies: *form determines form.*

Summary

1. A mercury drop in contact with a cylindrical surface takes the form of a flat elliptical disc. Increasing the volume of the drop causes an increase in the area and eccentricity of the drop but causes no appreciable increase in thickness. With constant drop volume, the larger the cylinder the less eccentric the fluid drop.

2. Analyses of blood cell form and capillary diameter in *Triturus* and *Pleurodeles* disclosed the following relationships. The red cell is circular if its area does not exceed that of the capillary. Eccentricity increases first in a curvilinear and then in a linear fashion as the red cell increases beyond the cross-sectional area of the capillary. Under conditions of essentially constant red cell area, eccentricity is inversely related to the cross-sectional area of the capillary.

3. Based on the experimental findings the following equation may be derived relating red cell area, capillary cross-sectional areas and eccentricity (a/b):

$$(a/b) - 1 = K(A_{\text{cell}}/A_{\text{cap.}}).$$

4. Evidence for the physical nature of the red cell was discussed in relation to the model system.

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